THE ABSORPTION OF NUTRIMENT FROM SOLUTION BY FRESHWATER MUSSELS

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THE ABSORPTION OF NUTRIMENT FROM SOLUTION BY FRESHWATER MUSSELS[1]

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THIRTY FIGURES (TWO PLATES)

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INTRODUCTION

In 1914 the author began a series of investigations upon fresh-water mussels designed to ascertain whether or not there could be found any direct proof, especially of a histological nature, of the correctness of Pütter's theory that animals living

[1] Published by permission of the Commissioner of Fisheries.

in the water use, in addition to 'formed' food, nutriment which is in solution in the surrounding medium. He wished also to put to the test Pütter's assumption that some of such nutriment is absorbed directly by the cells of the outer body walls, especially by those of the gills.

Pütter ('06) was the first seriously to advance the theory that food could be so taken. He based his conclusions in part on a comparison of the amount of carbon necessary for the maintenance of the organism with the amount furnished by the plankton. Pütter considered the latter too small for the needs of the animal and urged that it must use some carbon which is in solution in the water, resulting from the decay and disintegration of organic life. He further stated that the amount of material found in the alimentary canal is never large enough to supply the requisite quantity of carbon. Besides the alimentary canal, the uncutinized epithelium of the outer surface of the body, especially that of the gills, was thought to function in absorbing dissolved food. This process was considered to go on in addition of course to the digestion of 'formed' food by the alimentary canal. Pütter concluded that the above conceptions applied to Protozoa, Porifera, Echinoderms, Crustaceans, Mollusks and Fishes. He tested the matter experimentally in two ways: first by noting that goldfish and perch lived longer in solutions of asparagin, somatose and glycerin than in tap water: secondly by comparing the amount of oxygen needed to oxidize the lost weight of tissues of actinians, tunicates and fish while kept in their natural medium, with the amount of oxygen actually used. As the latter was found to be greater than the estimated quantity needed he concluded that the extra oxygen was used in oxidizing some food that had been taken from the water where it had been present in the form of a solute.

Lohman ('09) estimated the amount of plankton found in sea-water from various parts of the ocean and concluded that plankton comprised the main source of the food of aquatic forms. He quoted Henze's researches as showing that there is present in sea-water no appreciable amount of carbon compounds in solution.

Knorrich ('10), working with Daphnia, which lived 14 days in sterilized hay solution, concluded that nutriment was absorbed from the solution.

Kerb ('10) kept eels in sugar solutions and noted no diminution in the amount of sugar from day to day. He obtained similar results while working with Corethra larvae in sugar solutions. Also he found that Daphnia lost in dry body weight as rapidly in solutions of peptones as in tap water.

Wolff ('10), working with Simocephalus, found that it lived twice as long in bacteria-free water, which contained some dissolved carbon compounds, as it did in tap water. He made no observations as to body weight lost or gained.

Several American investigators of the question of the nutrition of the oyster have felt that the amount of material found in the alimentary canal was not large enough to account for the growth of the oyster to the relatively large size which it attains in the first two years of its existence.

Grave ('12) in particular suggested that in the oyster food may be taken in by means other than that of the alimentary canal.

Moore, Whiteley, Edie and Dakin ('12) investigated the rate of oxidation and the output of carbon dioxide by aquatic invertebrates in relation to the available food supply in sea-water. They also made chemical analyses of samples of sea-water from various regions. Their general conclusion was that sea-water does not contain any appreciable amount of dissolved organic matter capable of acting as a nutrient medium for animals living in it.

Lipschütz ('13) reviewed almost the entire subject including his own previously published experiments along that line and offered criticism of Pütter's work. Lipschütz noted that fish and eels when kept in nutrient solutions lost as much body weight as in tap water. He also thought Pütter had overestimated the amount of material in solution in the water and underestimated the carbon content of the plankton. His general conclusions are the opposite of those of Pütter.

Blegvad ('15) found that the invertebrates on the sea-bottom in Danish waters live on detritus and to a lesser extent on plankton. He does not corroborate Pütter's theory but on the contrary thinks that the latter did not allow enough for the quantity of detritus.

Esterly ('16) found that in pelagic copepods the amount of food that is indicated by the intestinal contents is 'surprisingly small.' He suggests however that it is probable that much of the food of these forms consists of organisms without shells and which do not leave recognizable remains in the intestine. Further careful investigation he feels is necessary before Pütter's theory is accepted.

Lund[2] found that if Bursaria are kept in a weak soap solution they will absorb fat from such solution through their body walls.

The present author, in 1915, published a paper in which were set forth the results of the earlier steps of his investigations. Fat was selected for the initial experiments as it is most easily traced histologically. Following the method of Lund, olive oil was saponified and the mussels kept in weak solutions of the resulting soap. In some cases the solutions were stained with Sudan III. Briefly stated, it was found that the mussels absorbed fat from the solutions to a marked extent. This was transported over the body by the plasma of the blood and by the corpuscles. Several of the experiments went far toward proving that some of the fat was absorbed directly by the cells of the gills, mantles and foot.

The present paper discusses the results of the continuation of the series of investigations. Further work was done on the absorption of fat using a commercially prepared soap instead of the saponified oil. The absorption of protein was studied somewhat extensively and some efforts were made to ascertain the facts in regard to the absorption of starch.

The author wishes to express his obligations to Dr. Caswell Grave, at whose suggestion the work was undertaken, for advice and aid given throughout the course of the investigations:

[2] Dr. Lund's results are not yet published.

to Dr. H. S. Jennings and Dr. R. E. Coker for their suggestions and interest in the work: to Dr. G. L. Houser for library facilities at the University of Iowa; and to Mr. A. F. Shira, Director of the Biological Station at Fairport, Iowa.

MATERIALS AND METHODS

The mussels upon which the investigations were carried out were individuals selected from the more common species found in the Mississippi River near Fairport, Iowa. Adult specimens were employed in the following experiments. Care was exercised to choose non-gravid mussels which were in a seemingly healthy condition, 'shoulder-raked' or hand collected individuals being used in preference to those dragged out of the water by 'crow-foot' hooks.

At Fairport soft water was used for the work with fat, for the reason that the soap was more soluble in it than in river water. For the experiments with protein and starch filtered river water was used. At the Johns Hopkins Laboratory, where the work concerned only protein and starch, either city tap water or Chattolanee spring water was used.

The mussels experimented with were kept in glass aquaria of about 5000 cc. capacity. Control individuals in filtered water were kept side by side with those in the solutions. The solutions were changed daily in most cases. In some of the experiments with protein the changes were made twice daily. By this procedure the protein was prevented from undergoing marked decomposition. As the mussels are accustomed to a current of water this method of manipulating the aquaria seemed to be the closest approximation to natural conditions which it was possible to obtain in the laboratory.

For the experiments with fat a 'non-alkaline' soap commercially prepared from olive oil was used. This was done to avoid the free alkali and oil present in the saponified olive oil employed in the earlier investigations. The strength of the solution used was 0.002 of one per cent. The tissues of various portions of the mussels experimented with were sectioned by the freezing

method, stained with Sudan III and mounted in glycerin. In some cases the soap solution was stained by dissolving Sudan III in it to the point of saturation. Sections of mussels which had been kept in such solutions were mounted directly after cutting.

A method of attacking the question of the absorption of protein was much more difficult to devise. Egg albumin was chosen as the most convenient protein with which to work. The white of one egg was stirred into 300 cc. of water and the mixture filtered. The soluble albumin passed through the filter leaving the insoluble globulin behind. (The presence of albumin in the filtrate could be demonstrated by the formation of a white precipitate on the addition of alcohol.) 50 to 100 cc. of the filtrate were used in 5000 cc. of water to make the solutions in which the mussels were kept. Some experiments were continued for considerable periods of time, thirty to sixty days, and histological evidence sought of differences in the condition of the cells of those individuals which had been in the solutions and of the control mussels. As the latter, being in filtered water, received little food it was thought that evidence of starvation might be detected by the appearance of the cells. In other cases the experiments were continued only a few days, the tissues of the mussels were fixed in absolute alcohol or other fixing fluids and sectioned in paraffin. These were studied both stained and unstained. Efforts were made to find a specific stain for the albumin. Cowdry ('14) has found that Janus green, besides staining mitochondria, stains albumin in the living cell. This fact was made use of and mussels were kept in solutions of albumin stained with Janus green. Sections of such individuals were made by the freezing method and mounted in glycerin. The paraffin method failed with such sections as the stain is washed out very readily by the alcohols. It was necessary to study the sections mounted in glycerin within the course of a day or two as the stain is also removed by the glycerin in a short time.

Soluble starch was made by boiling and weak solutions were used in which to keep the mussels. Sections of such mussels

were prepared both by the paraffin and by the freezing methods and efforts were made to detect starch in the cells by staining with iodine. Mussels were also kept in solutions of starch which had been colored blue with iodine. Spring water was used in this case as the tap water contained so much free chlorin that the blue color was removed from the solution.

Further details of the methods employed will be stated in connection with the observations recorded below.

OBSERVATIONS

Fat

The solutions made from the commercial soap were found to be more satisfactory than those used in the previous work with fat where olive oil was saponified with sodium hydroxide. In the solutions from the latter the mussels were found to throw off much mucus, no doubt owing to the free alkali present due to a slight excess of sodium hydroxide employed in the process of saponification.

The mussels threw off no appreciable amount of mucus while in the solutions prepared from the non-alkaline commercial soap. None died while in the solutions. There was no evidence of any toxic effect due to the soap solution.

The results were the same as those obtained in the previous work with fat. The mussels which had been kept in the solutions were found to contain much more fat than those not so kept. This fat was distributed in the form of droplets[3] in greater or less abundance in nearly all parts of the body. Figures 1, 2 and 3 represent portions of a specimen of an adult Quadrula ebenus which had been allowed to remain in a 0.002 of one per cent soap solution for five days. The fat, represented in the drawing by the heavy black dots, may be seen in the epithelial cells of the gill filaments, the ostia, the water tubes and in the corpuscles.

[3] The term 'dropiets' will in this paper be applied to the spherules of absorbed fat found in the tissues of the mussels experimented upon. These droplets usually were of diameters varying from less than one micron to 3 or 4 microns, though instances were found in which the diameter was as great as 7 or 8 microns.

Mussels were kept in soap solutions stained with Sudan III. A gill filament of such a mussel after it had remained in the solution for five days is shown in figure 4. Pink or orange colored fat droplets were found in great abundance in the epithelial cells of the filaments, ostia, water tubes and in the corpuscles. This fact furnishes direct proof that the heavy loading of fat found in the mussels which had been in the soap solutions was not due to the chance use of an extraordinarily fat individual. The colored fat could have come only from the stained soap solution.

Mussels with the valves wedged open were suspended over the solutions in such a manner that only the lower edges of the mantles were immersed in the solutions. The animal soon relaxed the foot so that the end of that organ also was covered. Control individuals were suspended in like manner over filtered water. The experiment was continued for forty-eight hours. Numerous fat droplets were found in the cells of the parts of the mantles and foot which had been immersed in the solution and none were discovered in similar tissues of the controls. Epithelial cells of the mantles and foot that had been covered by the solution are represented by figures 5 and 7, while figures 6 and 8 show cells of corresponding parts of the control muscles.

It was thought possible that in this case the presence of fat was due to fatty degeneration incident to the weakened or moribund condition in which the mussel might be supposed to be after having been suspended thus with the larger portion of its body in the air for forty-eight hours. Such a supposition was rendered negligible by the facts that the mussels in which the fat was found were apparently in a healthy condition when killed; that no fat was found in the controls and none in mussels which had been allowed to die while so suspended over filtered water.

Finally, a large mussel with the valves wedged open was placed over the edge of the aquarium in such a manner that the lower edge of one mantle only was immersed in the soap solution, the entire remainder of the body being in the air. After a period of forty-eight hours sections of the part of the mantle

which had been in the solution revealed many fat droplets in the cells on the side next to the body. No fat droplets were found in the cells of the corresponding part of the other mantle which had not been in the solution. Some of the cells of the mantle which had been in the solution are represented in figure 9, while figure 10 shows cells of the other mantle which had remained in the air. In all these cases in which the mussels were suspended in such a manner as to allow only a portion of their body to come into contact with the solution, the oral and anal openings were above the solution so that none of the solution could have entered the alimentary canal directly. In the case last mentioned, in which one mantle of the mussel was immersed in the solution and compared with the other mantle of the same mussel, this mantle not having been immersed, direct proof of the absorption of fat from the solution by the epithelial cells of the mantle is given. In all cases in which one mussel is used as a control for another the objection can be raised that by chance a naturally fatter individual was chosen to be kept in the solution. But that objection can not hold in this case as it is hardly supposable that corresponding parts of the two mantles of the same mussel would differ in fat content as markedly as indicated in figures 9 and 10.

Protein

The problem of the absorption of protein was first attacked by means of the 'starvation' method. Five mussels were kept in solutions of egg albumin and five in filtered water. Both experiments were started on the same day and at intervals one individual was removed from the solution and one from the water at the same time and their tissues fixed and sectioned. Bouin's fixing fluid was used for this work and the sections were lightly stained with haematoxylin and eosin. One pair of mussels was killed at the expiration of ten days, other pairs at the end of thirty, forty-five and fifty-eight days. One of the individuals in filtered water died on the twentieth day. The tissues of the mussels were compared by the study of the sections for the purpose of discovering any possible difference in the degree

of emaciation between those that had been in the solution and those that had not. The criteria taken into account in judging the degree of emaciation were shrinkage of the cells and loss of capacity of the cytoplasm of taking up the eosin stain. Many workers on starved tissues have described great shrinkage and loss of staining capacity on the part of fasting cells. Morgulis, Howe and Hawk ('15) found that the cytoplasm of the cells of a dog which had suffered extreme starvation took a much less pronounced eosin stain than the cells of normal tissues.

The cells of the mussel which had been in filtered water for ten days showed no appreciable signs of emaciation. Various portions of its tissues were compared with corresponding parts of those of the individual from the solution, special attention being given to the epithelial cells of the gills in all the starvation experiments. In making the tests with the eosin stain a slide holding the sections from the mussel which had been in the solution was placed back to back with a slide of the sections of the control and both were passed through the alcohols and eosin together. In this way it was certain that both were exposed to the action of the reagents for exactly the same length of time and that any difference found in depth of stain was due to a difference in the cells and not to a variation in the time the tissues had remained in the stain. In some cases sections from mussels to be compared were mounted on the same slide.

In the comparison of the mussels killed at the expiration of thirty days, marked differences were found between the cells of those that had been in the solution and of those which had been in filtered water only. These differences were especially noticeable in the cells of the gills. The cells of the mussels which had been in the solution were plump, unshrunken and took the eosin stain in a normal manner. The gill filaments of those from the filtered water were small and wrinkled. The cells were shrunken and took a much lighter eosin stain than did those of the mussels from the solutions. Figures 11 and 12 represent respectively filaments of the mussel which had been in filtered water and of the one which had remained in the solution of albumin. These figures show most clearly the shrinkage

and distortion of the cells, the differences in the depth of stain taken being less exactly represented by the stippling. In figures 13 and 14 are shown respectively filaments of a mussel which had been subjected to filtered water and of one which had been in the solution for fifty-eight days. The same differences were found in this case as in the former. The pair killed at the end of forty-five days also manifested these differences.

The above facts leave little doubt that the mussels in the solutions make use of the albumin as food. The mussel that died during the course of the experiment was one of the lot in filtered water and its death was probably due to starvation.

Efforts were made, however, to devise methods whereby the albumin might actually be seen in the cells. Experiments were carried out in which some mussels were kept in solutions of albumin and others in tap or spring water. Sections of those in the albumin solutions were mounted on slides with corresponding sections of individuals which had been in water only. These were stained with eosin or Bordeaux red and a study of the minute structure of the cytoplasm was made. In general the cytoplasm of the mussels which had been in the solutions was of a more granular nature than that of the other mussels. The granules were not only more numerous but many of them were larger. For example, in one case where 100 cc. of albumin in 5000 cc. of water had been used in an experiment of twenty-one days' duration the difference was quite marked. The effect of the fixing agents is to precipitate the proteins of the cell. Apparently there was a greater quantity of protein present in the case of the mussels which had been in the solutions of ablumin.

In an effort to discover whether or not the same result could be obtained if the alimentary canal were eliminated, the mouths of some of the mussels were plugged with dumbbell-shaped pieces of paraffin. In many cases the mussels died due to injury incident to the operation. If no injury was inflicted the mussels often lived for a week or two. If the mussel did not die in the course of one or two days it might be assumed that no injury had been inflicted and that the mussel would live for at least eight days, which was the length of time it was desired

that the experiments should continue. The cytoplasm was studied after staining with eosin or Bordeaux red with the same results as in the previous experiments. The cytoplasm of those mussels which had been in the albumin solutions was more granular, the granules being more numerous and larger, than in the mussels which had remained in water only. This was especially apparent in the cases of two pairs of mussels from an experiment extending over six days. The solution in which the mussels had been kept contained 100 cc. of albumin in 5000 cc. of water. The cytoplasm was stained, in the case of one pair of mussels, with eosin and in the other with Bordeaux red. In both cases there were more granules in the cytoplasm of the mussels which had remained in the solutions than in that of the others. If the presence of a greater quantity of granules in the cells of the mussels which had been in the albumin solutions may be taken as evidence of the presence of albumin, in these cases in which the mouths of the mussels were plugged the albumin could have been taken up only by direct absorption from the solution.

Considerable effort was expended in attempting to discover a specific stain for the albumin, so that its presence might be detected in a manner similar to that by which fat is revealed by means of Sudan III. Albumin was precipitated with absolute alcohol, caught on filter paper and mounts made of it, both while wet from the alcohol and also after dehydrating, clearing and embedding in paraffin. While unstained, the appearance of the albumin under the microscope was that of a quantity of granules of various sizes. Most of them were irregular in shape but now and then one of a spherical form was discernible. Some of the mounts were carried through one stain, some through another, the object being to find a dye that would stain albumin and yet not stain the general cytoplasm of a cell. No great degree of success was attained. The albumin took the various stains tried, including eosin, Bordeaux red, haematoxylin, Janus green and others. All of these dyes stain the cytoplasm of fixed cells except haematoxylin, which is not considered a cytoplasmic stain. Since, however, the only color this stain im-

parted to the albumin was that of a weak bluish-brown it proved to be of no great value. When fixed tissues of the mussels which had been in the albumin solutions were treated with haematoxylin, it was found impossible to distinguish any granules in the general mass of the cytoplasm that might have been stained with the haematoxylin. The cytoplasm in unstained fixed tissues is of a brownish color and it was difficult accurately to determine the color of the small opaque granules found in these sections.

Albumin solutions were stained by dissolving haematoxylin in them. Mussels were kept in such solutions and control individuals were allowed to remain in tap water in which haematoxylin had been dissolved. The mouths of the mussels were plugged as described above. The experiment was continued for five days. At the close of that time portions of the gills were fixed in absolute alcohol and embedded in paraffin. As absolute alcohol precipitates albumin and does not dissolve haematoxylin from tissues, it was considered a favorable fixative for this purpose. The sections were mounted unstained and search was made for blue granules which might have been taken up from the solution by the cells. The results of such a study were of a negative nature. The same difficulty was experienced as described above in regard to the fixed tissues. While some of the granules in the cells which had been in the solutions seemed to have a bluish tint, in general it was impossible to be certain of the matter. In other words, haematoxylin does not impart to albumin a stain sufficiently characteristic to permit of its being distinguished from the unstained granules about it.

However, another result was obtained which confirmed the results of an earlier experiment. The cytoplasm of the cells of mussels which had been in the solutions was of a much more granular nature than that of the cells of the individuals which had not. The granules were both more numerous and larger. Also, many could be seen adhering to the outer ends of the cells or entangled in the cilia. Appearances indicated that these granules were particles of albumin and that some had been taken into the cells. The gill filaments of mussels which had

been kept in albumin solutions thus stained are represented in figures 17 and 18. Figure 19 is a representation of the gill filaments of a mussel which had remained in water in which haematoxylin had been dissolved. The granules in the latter will be seen to be much less numerous than in the former and none can be observed clinging to the outer ends of the cells or to the cilia.

Finally a fact was discovered that led to more definite results. Cowdry, in a paper on mitochondria, makes mention of the fact that he has found that Janus green will, in the living cell, stain lecithin and albumin, the latter more heavily. As mentioned above the author had found that in fixed preparations Janus green stained the entire cytoplasm. An attempt was now made to determine whether or not Janus green might be employed as a means of detecting albumin that might have been absorbed by the cells of the gills or other parts of the outer body walls of the mussels.

Mussels were put into solutions made up 5000 cc. of water and 100 cc. of albumin stained with Janus green. Control individuals were placed in water in which Janus green had been dissolved. The mouths of these mussels were left open. The experiments were continued for eight days. At the expiration of that period the mussels were killed and their tissues were hardened in 10 per cent formalin for a few hours. As albumin is precipitated by formalin, this procedure would cause the precipitation of the albumin in any of the solution which had adhered to the outer surfaces of the cells in addition to the coagulation of what albumin might have been absorbed. In this way aggregates of albumin sufficiently large to be visible under the microscope would be formed and thus render it possible to trace this protein histologically. Sections were then cut by the freezing method and mounted in glycerin. The gill filaments of a mussel that had been in the stained solution are represented in figure 20. Numerous green granules of various sizes were found within the cells and within corpuscles in the blood vessels in the center of the filaments. The granules which had taken the stain are represented in the drawing by heavy black dots. In

some cases these green bodies were almost spherical. This was especially true of the larger granules. In general the diameter of the granules varied from less than one micron to that of three or four microns. Green colored granules were also found clinging to the outer ends of the cells or adhering to the cilia. Figure 21 shows the filaments of a mussel kept in the water and Janus green. In this case no green granules were found either within the cells or on their outer surfaces. In some cases, both in the mussels that had been in the solutions and in the controls, the entire cytoplasm of the cells of the outer ends of the filaments showed a pale green tint. The granules in question, however, took a much deeper stain and were readily distinguishable from the surrounding cytoplasm.

Similar experiments were carried out in which the mouths of the mussels were closed with paraffin plugs. Exactly the same results were obtained. In figures 22, 23 and 24 are shown gill filaments of a mussel which had been in the stained solution. The black dots represent green granules and spherules which were found within the cells and corpuscles and clinging to the outer ends of the cells. The mussels used as controls in these experiments had no definite green granules within the cells or on the outer ends, though the cytoplasm often exhibited a faint green tint, as did that of those which had been in the stained solutions. A drawing of the gill filaments of one of the control mussels would present the appearance of the filament shown in figure 21. The presence of the green tint found in some cases over the entire cytoplasm does not militate against the efficacy of the Janus green as a specific stain for the albumin. Sudan III, while it is a specific stain for fat, often imparts a reddish tint to the entire cytoplasm, but stains the fat droplets a much deeper hue.

Figures 25, 26 and 27 represent respectively portions of the palp, foot and mantle of a mussel which had been in the stained solution. The mouth of the mussel had been closed with a paraffin plug. Green granules were found in the epithelial cells of all three tissues.

The above results demonstrate that the cells of the outer body walls of the mussels have the power to absorb at least one protein, albumin, from the surrounding solution.

Starch

Soluble starch was made by boiling commercially prepared cornstarch in water. One gram of starch was used in about 400 cc. of water. The solution, after boiling, was allowed to settle and 50 or 100 cc. of the supernatant liquid used in making the solutions in which the mussels were to be kept. This method affords only a rough estimate of the amount of starch present, as the amount of starch that went into solution was not determined. However, qualitative results only were sought in these initial experiments. The supernatant liquid was proved to contain starch by testing with iodine. After the mussels had remained in the solutions for various lengths of time they were killed and sections of their tissues were made both by the freezing and by the paraffin methods. The sections were then placed in alcoholic solutions of iodine for different periods of time and an effort was made to find blue granules within the cells. The results were negative. The tissues as a whole often assumed a slight yellow tint from the influence of the iodine, but no granules were found that could be considered to have a blue color. As iodine stains dextrin or causes it to assume a red color, search was made for red particles in the cells. It was thought possible that the starch might have been more or less completely converted to sugar by the cells and that evidence of some of the incidental steps could be found. Very small red granules were found both in the tissues of the mussels which had been in the solutions and in those of the controls. These red particles were no doubt the pigment granules which impart to the tissues the pinkish hue observable in many specimens of Quadrula ebenus. They presented no difficulties in the work with fat which had been stained red with Sudan III, as the pigment was in the form of small, irregular, highly refractive granules readily distinguishable from fat droplets. Their presence offered some

complications, however, in the search for any red granules that may have been present owing to the conversion of starch to dextrin and to its subsequent staining with iodine. No satisfactory evidence of the absorption of starch was disclosed by the above methods.

The experiment of keeping the mussels in a starch solution which had been stained blue with iodine was then tried. The mouths of the mussels were plugged with pieces of paraffin as described above. After the expiration of four days the mussels were killed and sections were made of their tissues by the freezing method. The sections were mounted at once in glycerin without staining. In the cells of the gill filaments some granules were found that were blue in color, though the observation was made with difficulty owing to the opacity of the granules. Similar granules were found within the corpuscles and also clinging to the outer ends of the cells and to the cilia. They were quite irregular in shape and varied from one to three microns in diameter. The gill filaments of a mussel which had remained in the stained starch solution for four days are represented in figures 28 and 29. Figure 30 is a representation of the gill filament of a mussel kept in tap water.

These results present some evidence that starch may be taken up directly from a starch solution by the epithelial cells of the gills of the mussel. Since fewer experiments were carried out with starch than with fat and protein, the results can not be considered to be as conclusive as were those attained in the work with the latter.

Behavior of the corpuscles

An interesting point in connection with the behavior of the corpuscles of the blood was noted. In the author's previous paper dealing with the absorption of fat, mention was made of the fact that the corpuscles were often found pressed closely against the bases of the epithelial cells of the gills and that the corpuscles carried some of the fat droplets from the cells to the other parts of the body. Some of the fat droplets were appar-

ently transported while floating in the plasma of the blood. A description has been given above of the discovery of fat droplets, granules of albumin or of starch, as the case might be, in the corpuscles of the mussels which had been in the solutions. It was also found that the corpuscles, in addition to pressing closely against the bases of the cells of the gills, actually wandered out between the cells in such a manner that their entire surfaces were in touch with the cells, the corpuscle lying in a sort of cup between the contiguous cells. This phenomenon is illustrated in figures 15, 16 and 17. In great numbers of cases the corpuscles were found pushed out between the cells in such a way as to be entirely surrounded. In other instances a corpuscle might be observed partially surrounded as in figure 15, the process of fixation having killed it at the moment of entrance to or exit from between the two cells. Undoubtedly a position in which the corpuscle is entirely surrounded by the epithelial cells affords more surface for the exchange between the cells and the corpuscle not only of materials concerned in respiration, but also for the reception by the corpuscle of food material absorbed by the cells of the gill. Similar behavior of the corpuscles was not noted in parts of the body other than the gills.

SUMMARY AND DISCUSSION

The above results leave no doubt of the fact that mussels may make use of some kinds of food which are in solution in the water. A part, probably a small one, of such nutriment can be taken up directly by the outer epithelial cells of the body. As these animals are provided with a well developed digestive apparatus we may suppose that the absorbing power of these outer epithelial cells is a property that has been retained from a more primitive state in which the cell was less highly specialized and that this property is not a special adaptation correlated with the lack of a functional digestive system.

The nutriment taken up from the solutions by the mussels by means of the alimentary canal was no doubt absorbed by the cells lining the intestine in the usual manner in which 'formed'

food is absorbed after being rendered soluble by the digestive juices. In regard to the mechanism of the absorption of the foods by the cells of the outer body walls little can be offered.

In the case of the fats, numerous droplets which took the Sudan III stain were found closely attached to the outer ends of the epithelial cells of the gills or mantle. These droplets were probably the fatty acids, a small part of which were present in the soap solution owing to hydrolysis, by which process sodium hydroxide and the fatty acids would be formed; the remaining droplets were no doubt due to a slight acidity of the surface of the living cells resulting from the union of carbon dioxide from the cells with the water, forming carbonic acid. In the experiments in which olive oil was saponified no attempt was made to remove the glycerin from the mixture. Therefore free fatty acids, from the droplets just mentioned, and glycerin may have been absorbed separately and resynthesized to fat within the cells. This absorption may have been effected by phagocytic or amoeboid action of the cells or by solution in the plasma membrane and reprecipitation within the cell. In the case of the commercially prepared soap the objection might be raised that there was no glycerin present to be absorbed and reunite with the fatty acids after their entrance into the cells. However it is safe to assume that a moderate amount of glycerin was present in the soap from the fact that in the process of its manufacture the soap is 'salted out' of the glycerin, allowed to rise to the surface and removed while wet with the glycerin. Not all the glycerin is removed by the subsequent drying.

Again it is not certain that the drops taking the osmic acid stain as described in the author's previous paper or the Sudan III stain in the sections prepared by the freezing method were fats or only the fatty acids. It is known that osmic acid blackens or browns free fatty acids. It is possible that Sudan III also stains them. At any rate solutions made from the commercially prepared soap dissolve Sudan III readily and assume the characteristic red color which that stain imparts to fat. It is probable that both these stains for fat really affect the fatty acid radical only and that it is that radical which carries the Sudan

III into the cell. Therefore there may exist the possibility that the sodium oleate, stearate and palmitate from the soap may have entered the cell as such, the radical carrying the stain. The sodium may then have been separated leaving the radical free to unite with any glycerin which may have been absorbed.

In regard to the absorption of albumin it is necessary to assume either a power on the part of the cell to split the protein into its amino acids and the absorption of these as in the alimentary canal, or the direct taking in by the cells of the colloidal particles of albumin by means of something analogous to phagocytic action. The fact that the Janus green stain was carried into the cell offers some evidence that the albumin entered the cell as such without being previously split into the amino acids.

In the case of starch it seems probable also that the granules entered the cell by amoeboid or phagocytic action. The presence of the definite blue granules within the cells would somewhat oppose the theory of any conversion of the starch previous to absorption.

The present investigations demonstrate only the ability of the mussels to make use of nutriment which is in solution in the water by the twofold means cited above. They do not deal with the possible amount of nutriment present in various bodies of water in which aquatic animals are found. The investigations show that if dissolved material is present the mussels can make use of it. After the ability of the animal to make use of food in such form is proved, the question of whether or not, in any particular case, it does do so, depends upon the presence or absence of such food in the water. It is too sweeping a statement to assert that aquatic animals in general do not or can not make use of nutriment which is in solution in the water merely because no dissolved compounds are found in certain analyses of water taken from a more or less limited region. Nor is the fact of the presence of an amount of detritus, apparently adequate for the nourishment of a bottom-living animal, sufficient proof that all its nutriment is made up of such detritus.

In the case of mussels in particular it is very probable that considerable nutriment is in solution in the water in which they live. While no analysis of the water has been made thus far in the investigations, the Mississippi River, as everyone knows, drains a vast area of land from which refuse from decaying animal and vegetable material is collected in very great abundance. The dead bodies of aquatic forms of many sorts add to this supply. The mussels are bottom-living organisms and come into contact with decaying and dissolving organic matter which is lying on or being slowly moved along the substratum. Solutions or colloidal suspensions of the proteins must certainly be present in some abundance. As the water is slightly alkaline some of the fat from decaying organisms is probably saponified and thus distributed throughout the water instead of rising to the surface where it would be inaccessible to many forms.

In general the question of whether or not a particular aquatic animal absorbs nutriment from solution by means of the alimentary canal and the outer body walls probably depends on the presence or absence of dissolved substances in the water in which the animal in question lives. Fresh-water mussels can absorb nutriment which is in solution in the water and it seems very probable that other forms likewise possess this ability.

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PLATES

EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida; Leitz Oc. No. 4, Obj. No. 6 unless otherwise indicated.

PLATE 1

EXPLANATION OF FIGURES

1 Gill filament of Quadrula ebenus which had been in soap solution for five days. *A*, fat droplets; *B*, blood corpuscles containing fat droplets; *C*, chitinous rod.

2 Section from the same mussel as in figure 1, showing fat droplets in epithelial cells of *A*, ostium.

3 Epithelial cells of water tube of gill of same mussel as in figure 1.

4 Gill filament of Anodonta imbecillis which had been for five days in soap solution which had been stained with Sudan III. *A*, pink colored fat droplets; *B*, blood corpuscles containing pink fat droplets; *C*, chitinous rod.

5 Epithelial cells of mantle of Lampsilis ventricosa which had been suspended for forty-eight hours in such a manner that only the extremity of the foot and mantles were immersed in soap solution.

6 Epithelial cells of mantle of the control for figure 5, the mussel being suspended so that the foot and mantles were in water.

7 Epithelial cells of foot of the same mussel as figure 5.

8 Epithelial cells of foot of same mussel as in figure 6, control for figure 7.

9 Epithelial cells of left mantle of Lampsilis ventricosa. These cells are from the edge of the mantle which was immersed in soap solution, the remainder of the mussel being in the air.

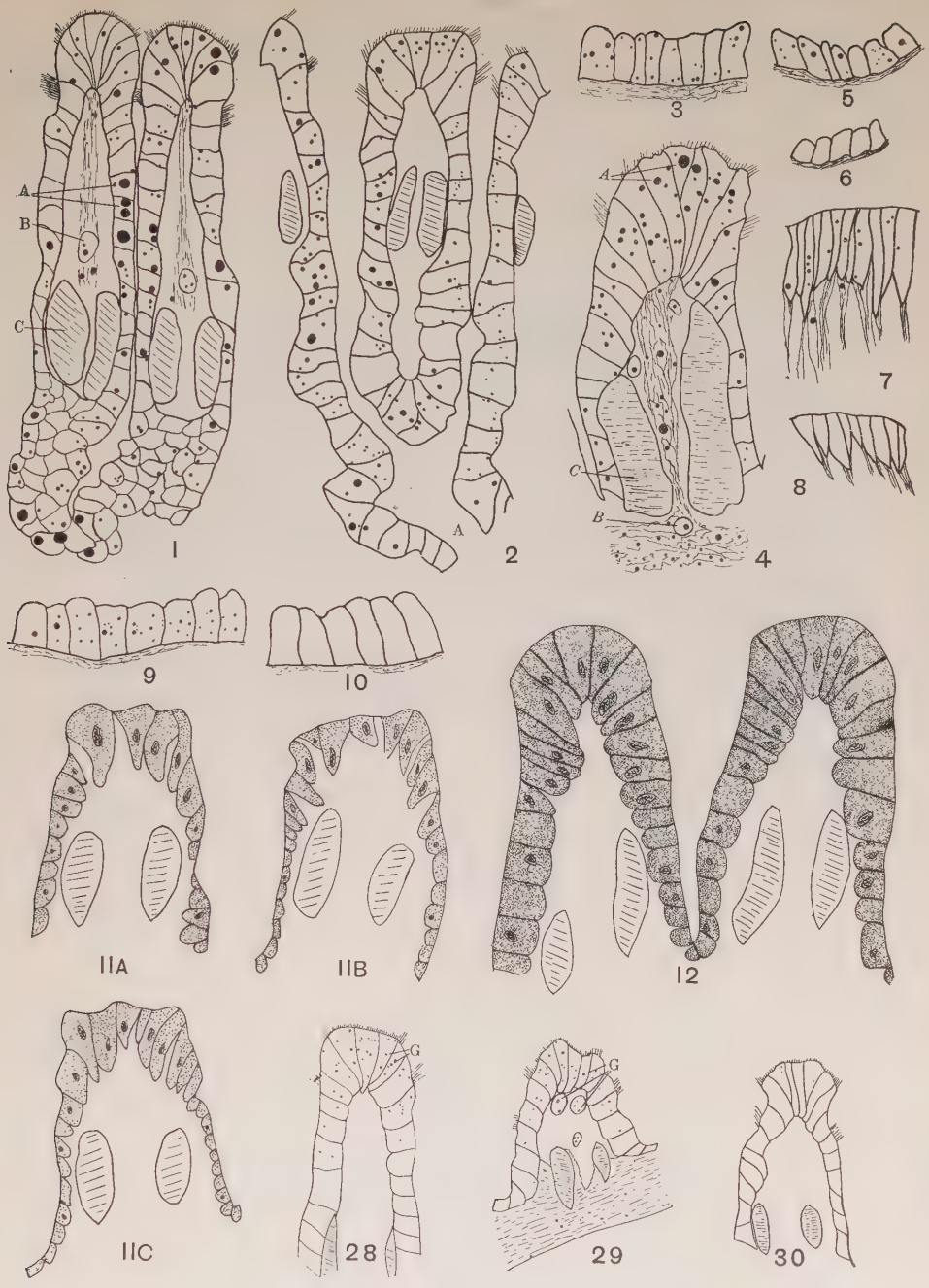
10 Epithelial cells of right mantle of the mussel shown in figure 9. These cells were not exposed to the solution.

11 *A*, *B*, and *C*, gill filaments of Quadrula ebenus which had been in filtered water for thirty days.

12 Gill filaments of Q. ebenus which had been in albumin solution for thirty days. The relatively heavier stippling in this drawing in comparison with that in figure 11 indicates the differenee in depth of eosin stain taken by the cells of the two mussels.

28 and 29 Gill filaments of Q. ebenus which had been for 4 days in starch solution stained blue with iodine. Mouth plugged. *G*, blue granules of starch.

30 Gill filament of Q. ebenus which had been in water only. Control for figures 28 and 29.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G
28
G
29
30

PLATE 2

EXPLANATION OF FIGURES

13 Gill filaments of Q. ebenus which had been in filtered water for 58 days.

14 Gill filaments of Q. ebenus which had been in albumin solution for fifty-eight days. The relative depths of stippling in this figure and the preceding one have the same significance as in figures 11 and 12.

15 and 16 Gill filaments of Q. ebenus showing corpuscles entirely surrounded by epithelial cells. *D*, corpuscles.

17 and 18 Gill filaments of Q. ebenus which had been in albumin solution with mouth plugged for five days. The heavy dots represent the granules found in the cytoplasm and adhering to the outer ends of the cells. Apochromatic homoegeneous immersion objective and compensating ocular.

19 Gill filament of Q. ebenus used as control for those shown in figures 17 and 18. Magnification as in figures 17 and 18.

20 Gill filaments of Q. ebenus which had been for eight days in albumin solution stained with Janus green. *B*, green granules of albumin; *C*, corpuscles containing green granules.

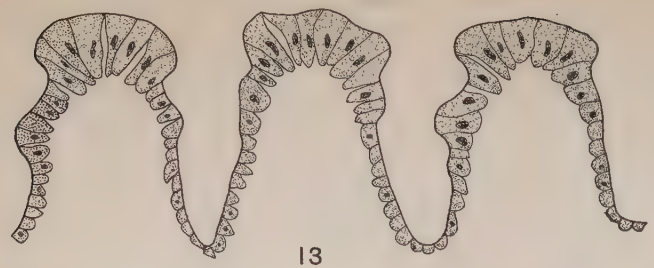
21 Gill filament of Q. ebenus which had been in water and Janus green for eight days. Control for figure 20.

22, 23 and 24 Gill filaments of Q. ebenus which had been with mouth plugged for eight days in albumin solution stained with Janus green. *E*, green granules.

25 Epithelial cells of palp of mussel shown in figures 22 to 24.

26 Epithelial cells of foot of mussel shown in figures 22 to 24.

27 Epithelial cells of mantle of mussel shown in figures 22 to 24. The black dots in figures 25 to 27 represent green granules of albumin.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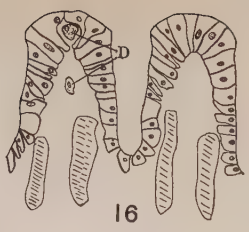

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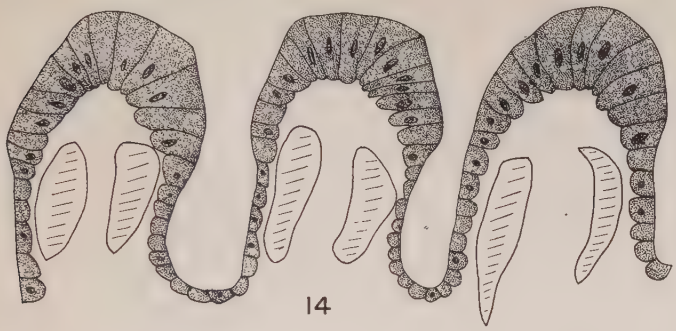
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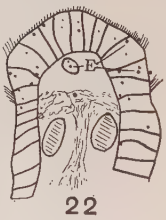

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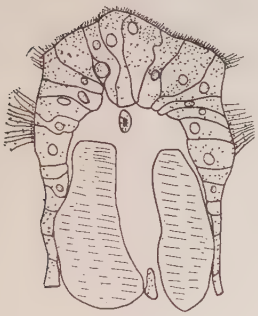
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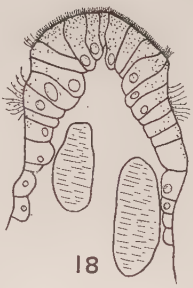
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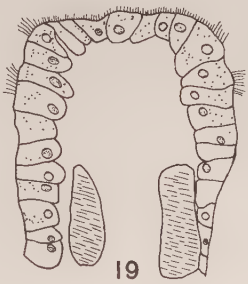
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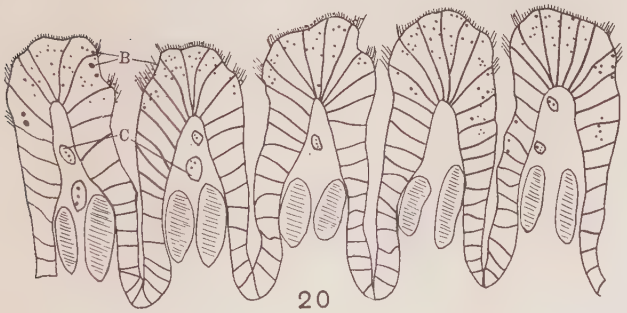

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VITA

Edward Perry Churchill was born at Allerton, Iowa, July 19, 1882. He was graduated from the Corydon, Iowa, High School in 1902 and entered the State University of Iowa in 1903, receiving the degree of A.B. from that institution in 1907. From 1907 to 1909 he served as Principal of the Corydon, Iowa, High School; 1909 to 1911 Principal of the Anamosa, Iowa, High School; and 1911 to 1912 Principal of the Washington, Iowa, High School. In 1912–13 he was graduate assistant in Zoölogy at the University of Iowa. In 1913 he entered Johns Hopkins University with Zoölogy as his major subject and Psychology and Physical Chemistry as minors. In 1913–15 he assisted Dr. Grave in Comparative Anatomy. He held a University Fellowship in Zoölogy at Johns Hopkins University during the year 1915–16.

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